NanoBiotec
Min samples, Max info

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PrimeFlow RNA Assays

Simultaneous Quantitation of multiple RNA and protein expression in each single cell

ABOUT PRIMEFLOW RNA ASSAYS

During stimulation, gene expression is differentially regulated in cells. Accordingly, RNA and protein expression kinetics of certain genes may vary temporally. Due to limitations of widely used techniques, RNA and protein levels cannot be measured simultaneously. PrimeFlow technology addresses this problem by facilitating the concurrent evaluation of messenger/micro/non-coding RNA with protein dynamics at the single cell level in response to stimuli.

HOW DOES THE ASSAY WORK?

PrimeFlow RNA assays can detect RNA and protein simultaneously in a variety of cell subsets. This assay employs fluorescence in situ hybridization (FISH) with branched-DNA signal amplification for the simultaneous detection up to four RNA targets. Further, RNA labeling can be combined with cell surface and intracellular fluorescent antibody staining adding a greater degree of specificity. A general work flow is as follows:

1. Treated and control cells or tissue samples are harvested and stained with viability dye and cell surface markers antibodies.
2. The cells are fixed and permeabilized.
3. The cells are stained with the antibodies for intracellular proteins (optional).
4. Cells are then incubated with RNA target probes, amplification reagents and label probes.
5. The stained and labeled cells are then acquired on the Cytoflex S flow cytometer.

ASSAY BENEFITS

- **Superior specificity**:
  - ✓ Measure RNA transcription at the single cell level.
  - ✓ Provide data for mechanistic insights.
- **Flexibility**: easily automated for use in routine compound screening
- **Simultaneous detection**:
  - ✓ Monitor RNA expression of several targets in the same cell type or in distinct cell subsets in a heterogeneous cell sample.
  - ✓ Evaluate protein and RNA kinetics
- **Standardized platform**: 96-well plate format using Cytoflex S flow cytometer.
- **Use of specialized reagents**: Assay conditions are optimized for accurate and reproducible data.

SAMPLE REQUIREMENTS

- **Minuscule samples**
- **Compatibility**: works with a variety of sample types such as cultured cells, PBMCs, etc

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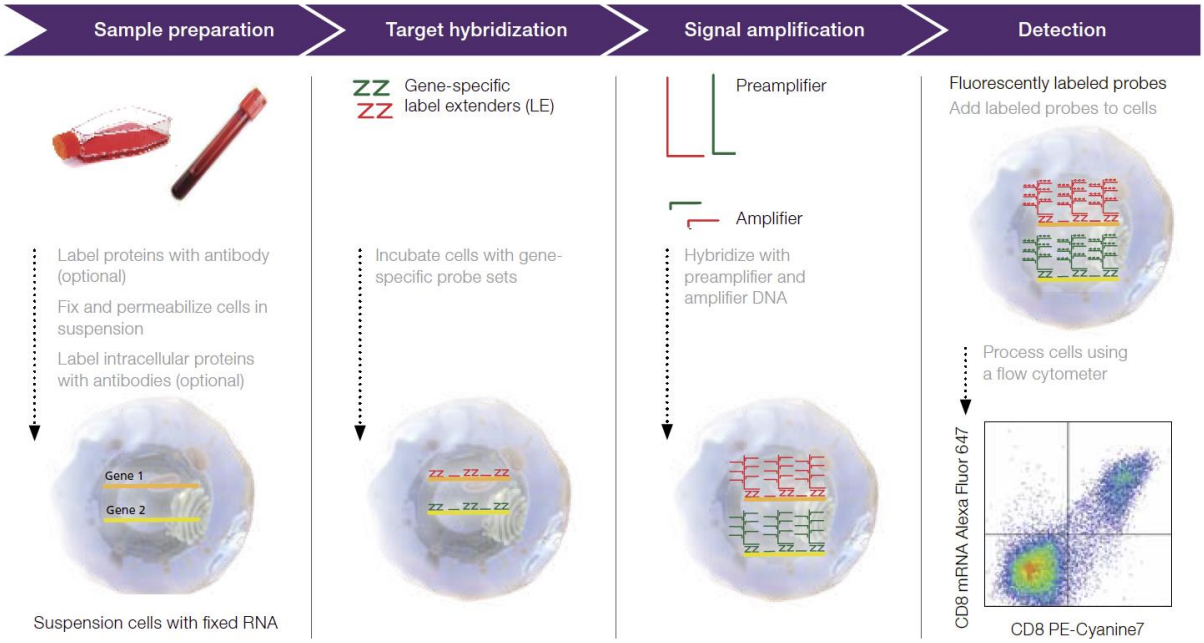
Four Main Steps of PrimeFlow RNA Assays



SERVICE FEATURES

- **High throughput**: 96-well format
- **Simultaneous quantitation:**
  - ✓ RNA and protein expression
  - ✓ Up to 4 RNA transcripts in one or multiple cell types
- **Detect cellular messenger, micro- and non-coding RNA**
- **Detect viral RNA in infected cells**
- **Analyze mRNA expression levels when antibody selection is limited**
- **Highly reproducible**
- **State-of-art platforms**: Cytoflex S flow cytometer
- **Extensive data analysis**
- **Timely data delivery**: 1-4 weeks or sooner, upon receiving test samples
- **20+ years of experience**: Expert data analysis and interpretation, high quality scientific and technical support

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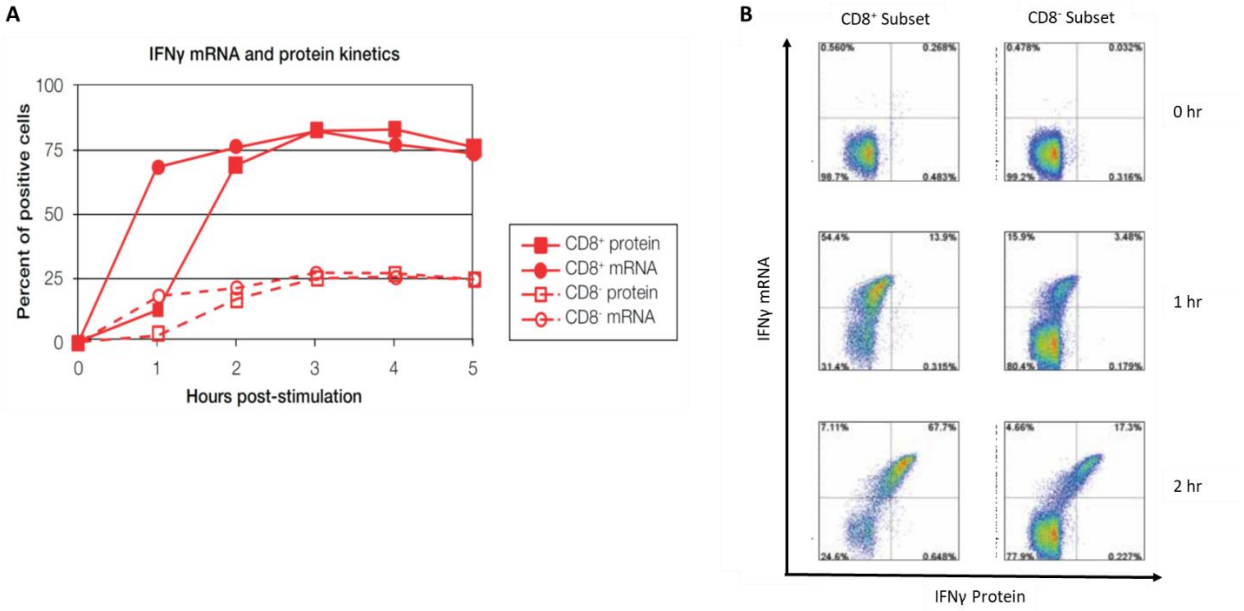

Example 1. Kinetics of IFN-γ transcription and translation measured by the PrimeFlow RNA Assay. Normal human peripheral blood mononuclear cells were stimulated with a Cell Stimulation Cocktail with protein transport inhibitors for 0–5 hours. Using the PrimeFlow RNA Assay, cells were fixed, permeabilized, and intracellularly stained with antibodies for CD8 and IFN-γ. Next, cells underwent a series of hybridization steps to label mRNA for IFN-γ. The time kinetics of IFN-γ mRNA and protein levels in viable $CD8^+$ and $CD8^-$ lymphocytic cells were evaluated.